A STUDY OF THE CORRELATION OF THE AGGLUTINATION AND THE FERMENTATION REACTIONS AMONG THE STREPTOCOCCI *

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Bacterial classification has passed through three distinct phases. First, we had the purely morphological classification based on size, shape, and form of the organisms and the colonies they produce. Later, when the inadequacy of a division founded on these characters alone was realized, various physiological reactions were added with the hope of obtaining a better grouping. Finally, pathogenic and immunity reactions were introduced in the differentiation of some groups. The later workers, however, did not always attempt to base their classifications on the correlation of these groups of characters, but recommended instead different systems depending on the types of reactions they happened to have observed. This created a consider-amount of confusion.

This state of confusion is especially notable among the streptococci. This group has from the very beginning been the subject of a great deal of controversy.

REVIEW OF THE LITERATURE

The discussion of the unity and multiplicity of the streptococci is too well known to bear repetition. Nor is it necessary to go further into the detailed history of the study of this group. Splendid critical summaries of the literature are given by Winslow,[1] and more recently by Hopkins and Lang.[2] In general, it will be noted that the first classification was a morphological one—longus, brevis, and media. It was soon found, however, that this separation was artificial and unreliable. Consequently Schottmüller[3] proposed a classification based on the action of these organisms on blood. Subsequently Gordon[4] and Andrewes and Horder[5] suggested a grouping based on fermentation reactions which promised to be scientifically tenable as well as practically valuable. This work was followed in this country by that of Winslow

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1. Jour. Infect. Dis., 1912, 10, p. 285.
2. Ibid., 1914, 15, p. 63.
3. München. med. Wchnschr., 1903, 20, p. 849.
4. Rept. Med. Off. to Local. Gov. Bd., Great Britain, 1902, 32, p 421.
5. Lancet, 1906, 171, p. 708.

and his co-workers[1] and Rogers,[6] who succeeded in bringing to light some valuable information on the streptococci of milk, cow and horse, and normal man. The pathogenic cocci, however, were not studied as carefully until recently when Hopkins and Lang[2] and, about the same time, Lyall[7] published thorough studies of this group. Hopkins and Lang used the fermentation tests only, while Lyall also utilized the hemolysin test, determined by a new volumetric method.

With the increasing volume of research a great body of apparently contradictory results has been published. However, a careful analysis of these data reveals the fact that the various workers, tho employing somewhat different technics, arrive at similar conclusions. Most authors agree that the streptococci generally ferment the sugars in a definite gradation, the simpler sugars being attacked first, then the complex, then the glucosid (salicin), and finally the alcohols. Organisms attacking a disaccharid generally ferment also the monosaccharids, and those fermenting a glucosid will also ferment the disaccharids. This has been aptly termed by Winslow the "metabolic gradient." There is also a general agreement that morphology, or length of chain, does not correlate with any other character. The value of the hemolysin test, on the other hand, is still undecided, tho it appears to be significant among the pathogenic streptococci. For the last mentioned group, Andrewes and Horder, Hopkins and Lang, and Lyall agree that salicin, raffinose, mannite, and inulin are the significant sugars, and that the group which generally ferments salicin (consequently also dextrose, lactose, and saccharose) but fails to ferment mannite, raffinose and inulin, and usually produces hemolysis is a definite species corresponding to the streptococcus pyogenes. Lyall uses the hemolysin test for the primary division, while the others use the sugars as the basis. A comparison of their tables reveals however a substantial agreement. Less certainty exists regarding the mildly pathogenic raffinose and mannite fermenters. These types have been vaguely classed as either viridans, salivarius, or fecalis, but as yet no satisfactory systematic division has been made.

For the non-pathogenic forms from human and animal origins, certain facts also have been definitely established. Winslow and Palmer,[8] confirmed by Fuller and Armstrong,[9] showed that lactose was significant in distinguishing the horse forms from those of human and bovine origins, as the former generally failed to ferment that sugar. The bovine forms are generally found to ferment raffinose, while the human ferment mannite.

None of the workers mentioned attempted to utilize the immunity reactions for the classification of this group. Aronson[10] and Marmorek[11] showed that the serum of one strain of pyogenic streptococcus may give a considerable amount of protection against other strains. They also showed that agglutinins produced by one strain will agglutinate other strains, usually in higher concentrations. These authors seem to think that while the agglutinuation reaction may be relied on to separate streptococci from pneumococci, it is of little value for differentiation of types within the group.

6. Jour. Ag. Research, 1914, I, p. 491.
7. Jour. Med. Research, 1914, 30, p. 487.
8. Jour. Infect. Dis., 1910, 7, p. 1.
9. Ibid., 1913, 13, p. 1.
10. Berlin klin. Wchnschr., 1902, 39, p. 1006.
11. Ibid., 39, p. 299.

Besredka and Dopter[12] used the complement fixation test on a series of scarlet fever streptococci without positive results. Faix and Mallein,[13] on the other hand, claim to have obtained cross fixation with nine strains of throat streptococci from cases of scarlet fever. Swift and Thro[14] studied the complement fixation and conglutination tests and found fixation specific for each of the five strains tested, while the latter test tended to bring out the group relationship.

Obviously, there must be some explanation for these conflicting results. It seemed likely that, by comparing the immunity reactions of the streptococci with their other properties, some light might be shed on this perplexing subject. In this paper, the following two points were taken up: Do the immunity reactions separate the pathogenic streptococci into distinct groups? Do the groups so formed correspond with those obtained by the use of the fermentative and other characters, i. e., is there a definite correlation between the immunity reactions and the other properties of these organisms?

Only cultures isolated from some pathogenic source were studied. In all, sixty strains were used. The tests employed were morphology; hemolysis; fermentation of lactose, saccharose, salicin, raffinose, mannite and inulin, and agglutination. Later, absorption tests were carried on parallel with the agglutinations, and in a few instances, complement fixation tests were made. A survey of the literature indicated however that the agglutination reaction was the one most likely to yield satisfactory results, and it was therefore used throughout the investigation.

METHODS

Morphology.—Smears were made from twenty-four-hour glucose broth cultures, and the average length of the chain was observed. My results are in accord with those of the other authors. In a general way, the salicin fermenters gave long chains, but frequently the chains of mannite fermenters were equally long. It was also noticed that the raffinose and mannite fermenters generally gave more abundant growths in glucose broth. The actual observations were not sufficiently uniform to warrant repetition here.

Hemolysis.—Tests for hemolysin production were made by streaking a loop-full of a twenty-four-hour culture of the organism grown on North's medium on the surface of a blood agar plate, containing 1 c.c. of defibrinated rabbit's blood to about 10 c.c. of agar. The

12. Ann. de l'Inst. Pasteur, 1904, 18, p. 373.
13. Presse méd., 1907, 15, p. 777.
14. Arch. Int. Med., 1911, 7, p. 24.

results were recorded under three heads: H, hemolysis; G, green colonies with or without slight hemolysis; and N, gray or brown colonies showing no action on hemoglobin. The results were not always constant, and in two or three cases it was uncertain in which group the organism belonged. On the whole, the reaction was uniform. An interesting observation, the constancy of which was not determined, is the fact that many of the raffinose fermenters gave green colonies accompanied by a slight zone of hemolysis, while the mannite fermenters usually gave a green to a greenish brown colony without any hemolysis. This reaction was merely noted and no significance can be claimed for it.

Fermentation.—The fermentation tests were made on two different occasions after an interval of about two months. The first test was made in meat infusion broth containing 1 percent peptone. It was found in agreement with Hopkins that a broth containing 2 percent peptone gave more uniform results, and so this medium was used for the second series of titrations. In both instances, the sugar broths were inoculated with a loop-full of a twenty-four-hour culture on North's medium. This was found more desirable than inoculations from a broth culture because no foreign, split protein substances of an unknown nature were introduced into the sugar broths, and also for the reason that the organisms have a greater viability and produce more abundant growth on the semi-solid than they do in the liquid medium. The sugar broths were thus seeded with a large number of vigorous cells, and consequently more constant results were obtained.

The non-uniformity of fermentative reactions attributed to the streptococci are due to the vigor of the inoculum (the culture) and the character of the new environment (the medium). It is well known that a vigorous culture inoculated into an unfavorable medium will give but sparse growth and that a weak culture even if inoculated into a favorable medium will often give no growth at all. It naturally follows that attenuated organisms, placed in an unsuitable environment, will generally fail to develop. A number of authors have demonstrated the fact that a culture, even in a fairly vigorous condition, when transferred to a fresh medium will pass through a definite period of retarded development before vigorous growth at the maximum rate begins. In the case of the streptococci, this is probably what happens. The organism, when placed in a medium

containing a glucosid or a higher sugar finds itself in an unnatural environment because the broth itself does not contain the substances favorable to active growth. Consequently, a period of "lag," as Penfold[15] names it, sets in during which the organism divides very slowly, while at the same time disintegration processes must be taking place. When weak cells or small numbers of vigorous cells are introduced into the medium containing the higher sugars, the cells die before they are able to exercise their latent power to utilize the sugar or alcohol for energy purposes.

This idea was confirmed in a number of ways. Inoculations were made into sugar broths from a broth culture, shown to contain living cells, by streaking on North's medium, and the former, when tested after twenty-four hours, were found to be absolutely sterile. Approximately the same amount of inoculum placed into salicin broth made up with 1 and 2 percent peptone, respectively, gave negative results in the former and active fermentation in the latter. On the other hand, two strains tested at thirty-day intervals for four months on the same batch of medium (kept tubed and capped on ice to prevent evaporation) gave uniform results. The tests were qualitative, but they all tend to show that the variable fermentations are frequently to be attributed as much to faulty methods as to the variability of the organisms. That the different cells of the same strain may vary somewhat in their power to ferment a certain sugar is an established fact.

The cultures were incubated for three days at 37 C. and the acidity titrated with n/20 NaOH, with phenolphthalein as an indicator. The results of the second series of titrations were similar to those of the first, except that in a number of instances salicin was fermented in the second series only. This is in all probability due, as stated above, to the more favorable character of the former medium.

Agglutination.—In all cases, the blood of the rabbits to be immunized was tested against the culture used in the immunization to ascertain the absence of agglutinins.

The first and second inoculations were made with killed twenty-four-hour glucose broth cultures. Subsequent inoculations were made at five-day intervals with increasing doses of living cultures. The size of the dose depended on the condition of the animal. Five to six

15. Jour. Hyg., Cambridge, 1914, 14, p. 215.

inoculations were generally sufficient to give a fairly high titer. In a number of cases, notably with cultures of the mannite fermenting groups, no immune substances could be detected in a dilution higher than 1:20, even after eight inoculations.

The method suggested by Hiss[16] of growing the culture in carbonate broth was found to be very satisfactory. The cultures can be stored on ice and used repeatedly. With this method, spontaneous clumping is reduced to a minimum.

The agglutinations were conducted in standard tubes using dilutions of the serum of 1:20, 1:50, 1:100, and 1:200. Controls of the homologous culture of salt water were always made. The tubes were incubated at 37 C. for two hours and then put in the ice chest for eighteen to twenty-four hours. The results were recorded as +, + +, + + +, and + + + + according as agglutination occurred in one, two, three, or four dilutions. Cultures that repeatedly clumped spontaneously in the salt water control were considered negative.

Parallel absorption tests were made with the last three of the four sera used. The results did not differ materially from those obtained with the agglutination test. A great deal of difficulty was experienced in absorbing the agglutinins, even with the homologous culture.

Complement Fixation.—In two cases, where the agglutinins in the serum were rather low, complement fixation tests were made with an emulsion of the organism and antiformin extract, respectively, as antigens. The results showed that the complement fixing substances were not more active than the agglutinins. The result is indicated by the following protocol:

Culture	Amount of Serum in c.c. (1-10)	Amount of Antigen in c.c.	Complement in c.c.	Inhibition	Agglutination 1:20	Agglutination 1:50
20	0.5	0.1	0.5	+ + + +	+	—
	0.2	0.1	0.5	+ +		
	0.1	0.1	0.5	+		

Control tests were made with antigen and serum alone, respectively, with negative results.

RESULTS

The results obtained with the various tests are shown in Table 1. With the exception of three or four cultures, the break between acid and non-acid producers is very sharp. All cultures producing 1 percent acid or over were considered positive; those below, negative.

16. Jour. Exper. Med., 1905, 7, p 223.

TABLE 1

Giving in Detail the Reactions of the Different Strains

No.	Source	Lactose*	Saccharose*	Salicin*	Raffinose*	Mannite*	Inulin*	Hemolysis			Agglutination with Serum			
								H	G	N	9	38	43	54
1	Blood, sepsis......	1.5 +	2.5 +	3.0 +	0.0 —	3.0 +	0.0 —	..	..	+	—	—	—	—
2	Blood, septis......	2.5 +	2.6 +	3.0 +	0.7 —	0.5 —	0.0 —	+	..	..	++++	—	—	—
3	Pus, abscess.......	2.8 +	2.7 +	0.8 —	3.0 +	0.4 —	0.0 —	..	+	..	—	—	—	—
4	Pus, mastoid......	2.9 +	2.8 +	2.8 +	0.4 —	0.6 —	0.0 —	+	..	..	+	—	—	—
5	Blood, sepsis......	1.5 +	4.0 +	3.6 +	1.0 +	0.4 —	0.0 —	..	+	..	—	—	—	—
6	Pericarditis	3.5 +	3.8 +	4.0 +	0.8 —	3.8 +	0.0 —	..	..	+	—	—	—	—
7	Blood (same patient as No. 3)	2.4 +	2.6 +	..	..	..	died	..	..	..	—	—	—	—
8	Antrum	2.5 +	2.8 +	2.8 +	0.9 —	0.6 —	0.0 —	+	..	..	—	+	—	—
9	Blood, sepsis......	2.5 +	2.9 +	4.0 +	0.6 —	0.2 —	0.0 —	+	..	..	++++	—	—	—
10	Peritonitis	+	3.2 +	3.2 +	0.7 —	3.6 +	0.0 —	+	..	..	—	—	—	—
11	Pus, abscess.......	+	3.0 +	3.0 +	0.3 —	0.4 —	0.0 —	+	..	..	—	—	—	—
12	Pus, knee abscess.	2.4 +	2.6 +	3.0 +	0.4 —	0.4 —	0.0 —	..	..	+	++++	—	—	—
13	Erysipelas	2.5 +	2.7 +	3.0 +	0.3 —	0.4 —	0.0 —	+	..	..	—	—	—	—
14	Blood, sepsis......	2.9 +	2.6 +	4.0 +	0.6 —	0.4 —	0.2 —	..	..	+	++	—	—	—
15	Septis, puerperal..	2.5 +	3.0 +	3.0 +	0.1 —	0.2 —	0.2 —	..	..	+	++++	—	—	—
-16	Sepsis, puerperal..	3.0 +	3.1 +	2.8 +	0.0 —	0.6 —	0.0 —	+	..	..	—	—	—	—
17	Blood, cellulitis....	2.5 +	3.2 +	2.8 +	0.1 —	0.6 —	0.1 —	+	..	..	—	—	—	—
18	Pus, abscess.......	2.8 +	3.8 +	2.6 +	0.1 —	0.2 —	0.0 —	..	+	..	—	—	—	—
19	Blood, sepsis......	+	+	+	—	—	—	+	..	..	—	—	—	—
20	Blood, sepsis......	2.6 +	2.6 +	3.2 +	0.4 —	3.4 +	0.0 —	+	..	..	—	—	—	—
21	Blood, sepsis......	2.4 +	2.9 +	3.5 +	0.5 —	0.2 —	0.0 —	+	..	..	+	—	—	—
22	Blood, sepsis......	1.5 +	2.5 +	3.0 +	0.2 —	0.0 —	0.0 —	+	..	..	—	—	—	—
23	Pleuritis	2.7 +	2.3 +	2.3 +	2.4 +	0.4 —	0.0 —	..	+	..	—	+	—	—
24	**Pleuritis**	2.4 +	2.5 +	3.8 +	2.5 +	0.0 —	0.2 —	..	+	..	—	+	—	—

* The numbers indicate the percent of normal acid.

TABLE 1.—(*Continued*)

No.	Source	Lactose*	Saccharose*	Salicin*	Raffinose*	Mannite*	Inulin*	Hemolysis			Agglutination with Serum			
								H	G	N	9	38	43	54
25	Peritonitis	2.9 +	3.2 +	3.6 +	0.0 —	0.0 —	0.1 —	+	..	..	—	—	—	—
26	Appendicitis	2.5 +	2.5 +	2.5 +	0.2 —	2.5 +	0.0 —	+	..	..	—	—	—	—
27	Pus, abscess.......	2.9 +	2.8 +	3.0 +	0.6 —	0.5 —	0.0 —	+	..	..	—	—	—	—
28	Pus, empyema.....	2.6 +	2.7 +	2.8 +	0.8 —	0.8 —	0.0 —	+	..	..	—	—	—	—
29	Acute tonsillitis (tonsil)	2.5 +	2.7 +	2.7 +	0.4 —	0.5 —	0.0 —	+	..	..	++++	—	—	—
30	Sputum, bronchitis	2.0 +	2.5 +	3.5 +	4.1 +	0.4 —	0.0 —	..	+	..	—	—	—	—
31	Pus, otitis media..	3.8 +	3.0 +	0.6 —	4.8 +	0.7 —	0.0 —	..	+	..	—	—	—	—
32	Acute tonsillitis (tonsil)	3.2 +	3.6 +	3.8 +	3.2 +	0.3 —	1.3 +	..	+	..	—	++++	—	—
33	Pus, abscess.......	3.0 +	2.5 +	2.4 +	0.6 —	0.4 —	0.0 —	+	..	..	++++	—	—	—
34	Liver abscess......	2.8 +	2.7 +	3.0 +	0.4 —	0.2 —	0.0 —	+	..	..	—	—	—	—
35	Pus, abscess prostate	3.5 +	4.0 +	3.8 +	0.6 —	3.5 +	0.0 —	..	+	..	—	—	—	—
36	Pus, abscess.......	2.5 +	3.2 +	3.0 +	0.4 —	0.5 —	0.0 —	+	..	..	++++	—	—	—
37	Stool	3.2 +	3.5 +	5.0 +	0.3 —	4.0 +	0.0 —	..	+	..	—	—	—	—
38	Tonsil, normal....	3.3 +	4.4 +	5.0 +	3.2 +	0.2 —	0.0 —	..	+	..	—	++++	—	—
39	Gum, pyorrhea....	4.0 +	2.8 +	1.2 +	1.0 +	0.3 —	0.0 —	..	+	..	—	—	—	—
40	Stool	3.2 +	2.9 +	5.0 +	0.3 —	3.9 +	0.0 —	..	+	..	—	—	..	—
41	....	3.3 +	3.6 +	4.2 +	2.8 +	3.1 +	0.0 —	..	..	+	—	—	—	—
42	Gland, scarlet fever	2.3 +	2.7 +	2.7 +	0.3 —	0.6 —	0.0 —	+	..	..	+++	—	—	—
43	Throat, scarlet fever	2.6 +	2.8 +	2.6 +	0.1 —	3.3 +	0.0 —	..	..	+	—	—	++++	—
44	Throat, scarlet fever	2.2 +	3.0 +	2.0 +	1.5 +	2.7 +	0.0 —	+	..	..	—	—	++	—
45	Liver, abscess.....	3.7 +	3.8 +	5.0 +	0.3 —	0.2 —	0.0 —	..	..	+	—	—	—	—
46	Cow, rappides.....	1.2 +	3.1 +	0.6 —	0.4 —	0.2 —	0.0 —	+	..	..	—	—	—	—
47	Cow, mastitis......	2.9 +	3.0 +	0.4 —	0.2 —	0.2 —	0.0 —	+	..	..	—	—	—	—
48	Nose, scarlet fever	2.0 +	2.6 +	2.9 +	0.7 —	0.7 —	0.0 —	+	..	..	+	—	—	

* The numbers indicate the percent of normal acid.

TABLE 1.—(*Continued*)

No.	Source	Lactose*	Saccharose*	Salicin*	Raffinose*	Mannite*	Inulin*	Hemoylsis			Agglutination with Serum			
								H	G	N	9	38	43	54
49	Throat, scarlet fever	1.3 +	2.4 +	0.7 —	2.4 +	0.0 —	0.0 —	..	+	..	—	—	—	—
50	Gland, scarlet fever	2.6 +	2.3 +	3.1 +	2.8 +	0.5 —	0.0 —	+	..	..	+	+	—	—
51	Septic sore throat, milk	2.6 +	2.9 +	2.8 +	0.7 —	0.0 —	0.0 —	+	..	..	—	—	—	—
52	Septic sore throat, gland	2.9 +	3.2 +	2.9 +	0.6 —	0.0 —	0.0 —	+	..	..	++++	—	—	—
53	Erysipelas	2.8 +	2.5 +	3.8 +	0.7 —	0.0 —	0.0 —	..	..	+	+++	—	—	—
54	Blood, endocarditis	3.0 +	3.1 +	3.5 +	3.0 +	3.0 +	0.2 —	..	+	..	—	—	—	++++
55	Blood, endocarditis	1.4 +	3.1 +	3.0 +	3.5 +	0.6 —	0.0 —	..	+	..	—	++	—	—
56	Blood, endocarditiss	3.4 +	4.0 +	3.5 +	4.2 +	0.3 —	0.0 —	..	+	..	—	++	—	—
57	Blood, endocarditis	3.2 +	3.0 +	3.9 +	3.2 +	3.6 +	0.0 —	..	+	..	—	—	—	—
59	Pus, abscess.......	2.3 +	2.5 +	2.8 +	0.0 —	0.1 —	0.0 —	..	..	+	—	—	—	—
60	Pus, abscess.......	2.2 +	2.6 +	3.0 +	0.3 —	0.1 —	0.0 —	..	..	+	+++	—	..	—

The interesting point in this study is the correlation of the agglutination reactions with other characters. Four agglutinating sera having titers between 800 and 1,000 were selected. These sera represented, respectively: A salicin fermenting, hemolytic strain (Strain 9); a raffinose fermenting, green colony producing strain (Strain 38); a strain fermenting salicin and mannite but not raffinose and producing no change on the blood (Strain 43); a strain fermenting salicin, mannite, and raffinose and producing green colonies on blood agar (Strain 54).

Tables 2-5 present a summary of the agglutination tests with these sera. The salicin serum agglutinated fifteen out of thirty-one of the salicin fermenting strains, twelve of them in dilutions of 1 : 50 or over, while it agglutinated only one raffinose fermenting strain in a dilution of 1 : 20, and failed to agglutinate any of the other fermentative types. Similar results were obtained with the raffinose serum, seven out of thirteen raffinose fermenters being agglutinated, four of them in dilutions of 1 : 50 or over. Of the others, only one salicin fer-

TABLE 2

AGGLUTINATION OF VARIOUS FERMENTATIVE TYPES BY THE SERUM PRODUCED BY A STRAIN FERMENTING SALICIN ONLY (STRAIN 9)*

Group	Number of Strains Tested	Number of Strains Agglutinated by a Dilution of the Serum			
		1:20	1:50	1:100	1:200
Salicin fermenters	31	15	12	11	8
Raffinose fermenters	13	1	0	0	0
Mannite fermenters	9	0	0	0	0
Mannite and raffinose fermenters	4	0	0	0	0

* This table shows that dilutions greater than 1:20 made the serum specific for salicin fermenters — tho even some of these were eliminated by this dilution.

TABLE 3

AGGLUTINATION OF THE VARIOUS FERMENTATIVE TYPES BY THE SERUM PRODUCED BY A STRAIN FERMENTING SALICIN AND RAFFINOSE*

Group	Number of Strains Tested	Number of Strains Agglutinated by a Dilution of the Serum			
		1:20	1:50	1:100	1:200
Salicin fermenters	31	1	0	0	0
Raffinose fermenters	13	7	4	2	2
Mannite fermenters	9	0	0	0	0
Mannite and raffinose fermenters	4	0	0	0	0

* This table shows that the serum was specific for raffinose fermenters in dilutions greater than 1:20, tho nearly half of the agglutinated strains were eliminated by the higher dilutions.

TABLE 4

AGGLUTINATION OF VARIOUS FERMENTATIVE TYPES BY THE SERUM PRODUCED BY STRAIN FERMENTING SALICIN AND MANNITE AND NOT RAFFINOSE*

Group	Number of Strains Tested	Number of Strains Agglutinated by a Dilution of the Serum			
		1:20	1:50	1:100	1:200
Salicin fermenters	31	0	0	0	0
Raffinose fermenters	13	0	0	0	0
Mannite fermenters	9	1	1	1	1
Mannite and raffinose fermenters	4	1	1	0	0

* This table shows that the serum was specific for the homologous strain except in one instance where a strain, fermenting both raffinose and mannite, was agglutinated in a dilution of 1:50, indicating that the types fermenting mannite alone and those fermenting mannite and raffinose are closely related.

TABLE 5

AGGLUTINATION OF VARIOUS FERMENTATIVE TYPES BY THE SERUM PRODUCED BY A STRAIN FERMENTING SALICIN, RAFFINOSE, AND MANNITE*

Group	Number of Strains Tested	Number of Strains Agglutinated by a Dilution of the Serum			
		1:20	1:50	1:100	1:200
Salicin fermenters	31	0	0	0	0
Raffinose fermenters	13	0	0	0	0
Mannite fermenters	9	0	0	0	0
Mannite and raffinose fermenters	4	1	1	1	1

* This table shows that the serum was specific for the homologous strain. Both this and the previous table tend to confirm the view that the mannite fermenters are less homogeneous than the other groups.

TABLE 6

CORRELATION OF FERMENTATION GROUPS WITH AGGLUTINATION*

Fermentation Group	Number of Cultures in Each	Cultures Agglutinated by Serum Produced by							
		Salicin Fermenter		Raffinose Fermenter		Mannite Fermenter		Mannite and Raffinose	
		Number	Per cent	Number	Per cent	Number	Per cent	Number	Per cent
Salicin	31	15	48.4	1	3.2	0	0	0	0
Raffinose	13	1	7.7	7	53.9	0	0	0	0
Mannite	13	0	0	0	0	1	15.4	1	7.7

* In this table, as well as in Table 7, all the cultures agglutinated by the different sera are included irrespective of the dilutions used, because this tends to bring out more strikingly the group relationships of the different strains.

TABLE 7

CORRELATION OF HEMOLYTIC GROUPS WITH AGGLUTINATION

Hemolytic Group	Number of Cultures	Cultures Agglutinated by Serum Produced by							
		Hemolytic Strain		Viridans Strain 1		Non-hemolytic Strain		Viridans Strain 2	
		Number	Per cent	Number	Per cent	Number	Per cent	Number	Per cent
Hemolysis	29	10	34.5	2	7.0	1	3.5	0	0.0
Green colony, slight or no hemolysis	17	0	0.0	6	35.3	0	0	1	5.8
No reaction....	12	5	41.7	0	0.0	1	8.3	0	0.0

menting strain was agglutinated and that in a dilution of 1:20. The mannite sera were specific for the respective homologous strains.

In no case was cross agglutination obtained in a dilution higher than 1:20. The two instances of such cross agglutination would indicate that this concentration does not bring out the specificity of the serum, tho a relatively greater number of homologous than heterologous strains were agglutinated in that dilution. Dilutions of

TABLE 8

CORRELATION OF FERMENTATIVE CHARACTERS WITH OTHER PROPERTIES

Group	Author	Number of Cultures Used	Sources												Size of		
			Suppuration		Septicemias		Acute Throat Infections		Endocarditis, etc.		Chronic Throat, etc.		Saprophytes		L.		M.
			Number	Percent	Number	Percent	Number	Percent	Number	Percent	Number	Percent	Number	Percent	Number	Percent	Number
Salicin fermenting strains	K.	31	14	47	12	40	3	10	0	0	0	0	1	3	25	48	6
	H.	52	22	42	10	19	5	10	4	8	8	15	3	6			
	L.	100	26	28	26	28	29	32	1	1	0	0	10	11			
Average	....	....	84 percent						16 percent								
Raffinose fermenting strains	K.	13	2	15	2	15	0	0	4	31	2	15	3	23	8	40	4
	H.	20	1	5	0	0	0	0	4	20	6	30	9	45			
	L.	55	1	3	1	3	13	39	1	3	4	12	14	52			
Average	....	....	30 percent						70 percent								
Mannite fermenting strains	K.	13	3	23	3	23	0	0	3	23	0	0	4	30	..	..	2
	H.	15	4	26	1	7	0	0	5	34	0	0	5	33			
	L.	37	0*	0	7	47	2	13	3	20.0	2	13	1	7			
Average	....	....	46 percent						54 percent								

* The grouping as to source is admittedly arbitrary and may not meet the approval of pathologists, but it is convenient for my purpose. Under acute throat infections are included septic sore throat and acute tonsillitis. Under endocarditis are classed also rheumaticus strains and those from pyorrhea. Under chronic throat are included chronic tonsillitis, bronchitis, and mild inflammations. In the saprophyte group are included all strains from normal throats, milk, etc. Under the heading, Size of Chain, L = longus; M = media; B = brevis. Under Hemolysis, H = hemolysis; G = green colony or

the sera of 1:50 or over are specific for the group; no cross agglutinations were obtained.

The correlation between agglutination and fermentative and hemolytic powers, respectively, is shown in Tables 6 and 7. At first sight the agreement is not very striking. Even if the non-specific agglutinations (agglutinations in a dilution of 1:20) are included, a correlation of only 50 percent is obtained with two of the fermentative groups and of only 35-40 percent with the hemolytic groups. A closer

analysis brings to light some interesting points. The serum produced by the salicin fermenter agglutinates only cultures of the same fermentative characters, except in one instance where slight agglutination was obtained with a raffinose fermenting strain. The same holds true of the serum produced by the raffinose fermenter, which agglutinates members of its own division. The sera produced by the mannite fermenting strains, on the other hand, remain practically specific for

TABLE 8
CORRELATION OF FERMENTATIVE CHARACTERS WITH OTHER PROPERTIES

Chain			Hemolysis						Agglutination								Fermentation	
M.	B.		H.		G.		N.		S +.		R +.		S +. M +.		S+, M+, R+		Salicin Percent.+	Raffinose Percent +
Per-cent	Num-ber	Per-cent	Num-ber	Per-cent	Num-ber	Per-cent	Num-ber	Per-cent	Num-ber	Per-cent	Num-ber	Per-cent	Num-ber	Per-cent	Num-ber	Per-cent		
11.6	18	34.6	23	74	1	3	6	19	15	48	1	3	0	0	0	0	100	0
			43	82.5	—	—	7	13.4	—	—	—	—	—	—	—	—	100	0
			74	74	16	16	20	20	—	—	—	—	—	—	—	—	100	0
				77		9.5		17.4									100	0
																		Mannite Percent +
20	8	40	1	7.7	11	84.7	1	7.7	1	7.7	7	54	0	0	0	0	77	0
			—	—	—	—	—	—	—	—	—	—	—	—	—	—	60	0
			1	2	38	68	16	29	—	—	—	—	—	—	—	—	73	0
				3		72		25									70	0
																		Raffinose Percent +
14	12	86	4	31	5	38	4	31	0	0	0	0	2	15	1	8	100	30.7
			3	20	..	..	11	73	—	—	—	—	—	—	—	—	93	20
			9	24	14	38	14	38	..	..	..	..	..	..	.	..	78	13.5
				26		38		36									86	18

methemoglobin reaction; N = no changes in the hemoglobin. The column showing size of chain is included to bring out the absence of any definite correlation between this character and the others.

In all cases, the percentages are determined on the basis of the number of cultures used in the particular test. This does not always bring out the true relationships, but this is a difficulty one generally has to contend with in comparing the results of different authors. Where comparable data exists, as in the case of Lyall's and my own hemolytic results, a close agreement is noted between the fermentation reactions and the source of the salicin fermenters.

their respective homologous strains. Therefore, while the agglutination reaction is apparently too specific for the differentiation of broad groups and not all the cultures of the same fermentative characters are agglutinated by their respective sera at the same time, the fact that a serum of one fermentative type was capable of agglutinating only strains of that particular group and not the others would indicate that the fermentation reactions tend to divide the streptococci into broad, distinct species. It is likely that by means of the agglu-

tination reaction these fermentative species may be further subdivided into varieties, similar to those obtained by Dochez and Gillespie among the pneumococci.

The correlation of agglutination with hemolysis is not so marked as that with fermentation. Thus 41 percent (five strains) of the indifferent strains were agglutinated by the serum of the hemolytic strain, and only 8 percent (one strain) by that of the indifferent strain. On the other hand, the agglutination correlation within the groups was about 35 percent for hemolytic and viridans strains, respectively. It seems that a primary division on the basis of hemolysis, as suggested by Lyall, is not warranted by the actual biologic relationships of the groups as indicated by the agglutination tests. Of course, it is possible that a better correlation might be obtained with his quantitative procedure.

On the whole, it is evident that the agglutination tests shed a considerable amount of light on the significance of the fermentative properties of the streptococci and on the importance of certain sugars for their differentiation into broad, specific types. The salicin group, as pointed out, has already been recognized by most workers as a well-defined, specific type. My observations are entirely in accord with these results. There is less agreement on the definiteness of the raffinose and mannite types. The agglutinations indicate that the organisms fermenting raffinose and not mannite constitute a fairly definite group. The mannite fermenters, on the other hand, form the center of a variable and ill-defined group, including mainly the saprophytic or mildly pathogenic forms.

The plausibility of this classification is strengthened when one recasts the data obtained by Hopkins, Lyall, and myself so as to bring out the correlation between the three fermentative types suggested and the various other characters. These results are shown in Table 8. There is a very substantial agreement between the results obtained by the different authors, despite the fact that non-uniform methods of recording them renders them at times incomparable. Hopkins, for instance, gives full data as to source and length of chain of his strains but fails to mention the methemoglobin, or green colony reaction. Lyall, on the other hand, gives full information as to the source of his salicin fermenting strains but furnishes meager data concerning the origin of the others. Nevertheless, certain facts stand out prominently. Thus, of the salicin fermenters 84 percent of which were

obtained from suppuration, septicemia, and acute throat infection, 77 percent hemolized blood, while only 9 percent produced green colonies. Of the raffinose fermenters, only 30 percent were found in acute infections and of these about two-thirds were from acute throat conditions. Seventy percent came from subacute and chronic infections, or saprophytic sources. Hemolysis was rare, while 72 percent produced green colonies. About 70 percent of these strains also fermented salicin. The mannite group again stands out as indefinite. The strains divide about equally between acutely and mildly pathogenic and saprophytic types. Tested on hemoglobin, they present almost equal numbers of hemolytic, viridans, and indifferent types. About 86 per cent of the strains also ferment salicin, while only about 20 percent attack raffinose. It seems to me that the evidence tends strongly to corroborate the conclusions arrived at from the study of the agglutination reactions.

SUMMARY AND CONCLUSIONS

Sixty strains of streptococci from various pathological conditions were studied with respect to their agglutinative and fermentative properties.

The agglutination reaction was not found to separate the streptococci into large groups. However, by its correlation with the fermentation reactions, the probable relationship of these types is indicated.

The agglutination tests tend to show that a division of the streptococci on the basis of hemolysis is not warranted, whereas a separation according to the fermentation reactions appears to coincide more closely with their natural relationship.

The groups suggested are:

Str. pyogenes.—Salicin fermenters, which do not ferment raffinose or mannite, are generally hemolytic, and strongly pathogenic.

Str. salivarius.—Raffinose fermenters, usually ferment salicin but do not ferment mannite, generally produce a green colony on blood agar, and usually cause subacute and chronic infections.

Str. fecalis.—Mannite fermenters, generally ferment salicin, rarely ferment raffinose, and are variable in their reaction to blood and in their pathogenicity.